Does Chronic Smoking Influence Fibrinolytic Potential in Type 1 Diabetes Mellitus?

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Tissue-type plasminogen activator (t-PA) and plasminogen activator inhibitor (PAI-1) were studied in 18 smokers and 18 closely matched non-smokers, all of whom had Type 1 diabetes mellitus (DM). None of the patients had advanced complications of diabetes. The t-PA and PAI-1 antigen levels were measured in plasma before and after venous occlusion, and were normal in Type 1 diabetes regardless of smoking status. Platelet PAI-1 levels were also measured and were found to be normal both in smokers and non-smokers. In smokers with Type 1 DM, plasma PAI-1 was significantly correlated with triglycerides. The normal fibrinolytic potential found in smokers with diabetes contrasts starkly with the significantly elevated plasma PAI-1 reported in smokers without diabetes. In smokers, triglycerides may effect low levels of PAI-1 release into plasma; this process may be significantly augmented in the presence of smoking-induced insulin resistance. The lack of endogenous insulin release in Type 1 diabetes may obviate the characteristic rise in plasma PAI-1 found in smokers who do not have diabetes.

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Introduction

The possible contribution of impaired fibrinolysis to the pathogenesis of thrombotic arterial disease has received increasing attention in recent years. Elevated plasma levels of plasminogen activator inhibitor type 1 (PAI-1) have been described in a variety of conditions associated with thrombosis.[1–3]

Type 1 diabetes mellitus (DM) is associated with a significantly increased mortality attributable to cardiovascular disease.[4] A wide variety of haemostatic abnormalities have been described in the condition[5,6] and may predispose toward the increased risk of coronary artery disease. The fibrinolytic enzyme system has been extensively studied in Type 1 DM but results have been conflicting (reviewed by Gough and Grant[7]). However, difficulties in interpretation of the existing literature include differences in methodology and the fact that several studies did not control for the effects of cigarette smoking.

We recently demonstrated that plasma PAI-1 is significantly elevated in cigarette smokers without clinical evidence of arterial disease.[8] We were interested in studying the effects of smoking on fibrinolysis in patients with uncomplicated diabetes in order to determine whether a similar pattern is observed to that in the population without diabetes, and whether abnormal fibrinolysis is detectable before the evolution of advanced complications in Type 1 DM.

We and others have previously demonstrated that most circulating PAI-1 (over 90 %) resides in platelets,[9,10] albeit largely in an inactive form. A number of qualitative and quantitative platelet abnormalities have been described in Type 1 DM.[11,12] We therefore aimed to examine the effects of smoking on the fibrinolytic potential of plasma and platelets from patients with Type 1 DM, without evidence of advanced microangiopathic complications.

Patients and Methods

Patients

Patients were identified from the computerized registry held at our diabetes clinic and were invited to participate in the study if they: were aged between 25 and 40;[13,14] had Type 1 DM; attended the clinic regularly; had no record of complications of diabetes at their last attendance (including normal fundoscopy through dilated pupils at least once during the previous year). Patients were

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eligible for the smoking group if they had smoked at least 15 cigarettes per day for at least 5 years and for the non-smoking group if they had never smoked. Wherever possible, the smoking history was corroborated by a partner or friend.

All patients attended for study in the morning, with smokers being asked to abstain from smoking from midnight the night before.[15] Patients followed their usual morning routine in terms of morning insulin and breakfast; fibrinolysis is not significantly altered immediately following exogenous insulin[16] or meals.[17] A medical history was taken and physical examination performed. All patients supplied a mid-stream sample of urine (MSU) and a sample for dipstick urinalysis.

Exclusion criteria for the study included: symptoms of hypoglycaemia on the morning of attendance; history suggestive of claudication or sensory impairment; history suggestive of ischaemic heart disease or of deep venous thrombosis; pregnancy; use of aspirin, non-steroidal anti-inflammatory drugs or the oral contraceptive pill; hypertension (defined as mean systolic blood pressure $\geq$160 mmHg and or mean diastolic blood pressure $\geq$90 mmHg); clinical absence of any of the peripheral pulses; evidence of retinopathy greater than grade 1; clinical evidence of sensory neuropathy or abnormal reflexes; anything greater than trace protein or trace blood on dipstick urinalysis; evidence of urinary tract infection on MSU; serum creatinine above the local reference range; clinical evidence of intercurrent illness.

Thirty-six patients (18 smokers and 18 non-smokers) satisfied the entry criteria. Two patients had less than three microaneurysms per fundus at fundoscopy. No other patients had evidence of retinopathy. A trace of haematuria was noted on urinalysis in one non-smoker and three smokers. A trace of proteinuria was found in three non-smokers and two smokers. All patients gave informed, written consent. The study was approved by the local Ethics Committee.

Protocol

The protocol employed has been described in detail previously.[8] In brief, blood samples were taken between 8 am and 10 am, in view of the known diurnal variation in fibrinolysis.[18] Samples for t-PA and PAI-1 estimation were collected onto an anticoagulant/antiplatelet mixture (Diatube H, Diagnostica Stago, Asnières-sur-Seine, France).[19] A standard venous occlusion test was then performed.[8] We have previously shown that the rise in platelet count accompanying venous occlusion closely reflects the haemoconcentration invariably associated with the test[8] and therefore a rise in platelet count was used to correct for haemoconcentration in this study. Standard techniques were used to prepare platelet rich plasma (PRP) and platelet free plasma (PFP).[9] PAI-1 in PFP represents the plasma pool of PAI-1, whilst subtracting PAI-1 in PFP from PAI-1 in PRP gives the platelet pool of PAI-1. Dividing the platelet pool of PAI-1 by the platelet count in PRP gives the level of PAI-1 per platelet.

Assays

T-PA antigen was assayed by enzyme-linked immunosorbent assay (ELISA) as described by MacGregor *et al.*[20] PAI-1 antigen was assayed by ELISA using purified PAI-1 as standard. The PAI-1 ELISA[21] was modified as described previously.[9] Biochemical and haematological parameters were measured using standard laboratory techniques.

Statistics

When comparing variables from different groups, the Mann-Whitney U test was used. Confidence intervals for differences between medians were calculated as described by Campbell and Gardner.[22] Spearman's rank correlation test was used when variables were tested for correlation.

Results

Baseline Characteristics of Patients

The smoking and non-smoking groups were closely matched for all measured baseline variables (Table 1). The smoking group had an average estimated cumulative exposure to cigarettes of 18.5 pack-years (range 7–28.8).

T-PA and PAI-1

No significant difference was found when comparing t-PA or PAI-1 antigen levels in non-smokers and smokers,

Table 1. Clinical, metabolic and anthropometric characteristics of smokers and non-smokers

	Smokers	Non-smokers
n	18	18
M:F ratio	14:4	15:3
Age (yr)	33 (25–39)	33.5 (26–39)
Duration of DM (yr)	4 (1.5–10)	6.5 (1.5–24.0)
Insulin (U day^{-1})	63 (34–94)	59 (25–108)
BMI (kg m^{-2})	24.9 (20.1–33.1)	26.1 (19.0–29.2)
Blood pressure		
Systolic (mm Hg)	125.5 (109–138)	122 (107–147)
Diastolic (mm Hg)	70 (60–86)	73 (50–87)
Glucose (mmol l^{-1})	15.2 (3.6–21.8)	13.2 (2.3–22.7)
HbA_{Ic} (%)	7.4 (5.1–9.7)	6.8 (3.8–9.6)
Triglycerides (mmol l^{-1})	1.16 (0.41–2.43)	0.81 (0.38–2.64)
Cholesterol (mmol l^{-1})	4.7 (3.3–7.2)	4.6 (3.4–6.8)
Fibrinogen (g l^{-1})	2.8 (1.9–4.0)	2.5 (1.7–4.2)
Albumin (g l^{-1})	40.5 (38–43)	41 (37–46)

Results expressed as median and range.

either before or after venous occlusion (Table 2). For t-PA pre-venous occlusion, the 95 % confidence interval for the difference in medians was from −1.0 to +1.8 ng ml^{-1}, and for PAI-1 from −9.5 to +3.6 ng ml^{-1}. The increase in t-PA after venous occlusion exceeded that due to haemoconcentration in both groups, but the increment in each was similar, with no statistically significant difference observed. No significant difference was found between the levels of platelet PAI-1 in the two groups.

Correlation between t-PA, PAI-1 and baseline variables

Plasma t-PA and PAI-1 levels were closely correlated both among non-smokers ($r = 0.61$, $p<0.05$) and smokers ($r = 0.63$, $p<0.01$). Among the smoking group, serum triglycerides correlated significantly with plasma PAI-1 ($r = 0.49$, $p<0.05$) and *t*-PA ($r = 0.54$, $p<0.05$). Neither PAI-1 nor t-PA correlated significantly with triglycerides in the non-smoking group.

Discussion

Our findings show that chronic smoking has little, if any, effect on the levels of t-PA or PAI-1 in patients with Type 1 DM of relatively short duration and without advanced microangiopathy. A previous study has described significantly elevated PAI activity in smokers with much longer histories of diabetes, many of whom had varying degrees of diabetic complications[23] which may have affected fibrinolysis.[24–26] Moreover, in that study,[23] as with several previous studies on fibrinolysis and diabetes, blood was collected onto citrate alone, raising the possibility that some of the measured PAI in plasma had leaked from platelets *in vitro*.[19]

The current study was designed to minimize the potential for diabetic complications to confound any observed effect of smoking on fibrinolysis. The finding of essentially normal t-PA and PAI-1 antigen levels in Type 1 DM patients without advanced complications broadly supports the data generated by groups who have subdivided patients according to the degree of diabetic complications,[25,27–29] although one group has described significantly reduced t-PA and PAI-1 antigen levels in uncomplicated diabetes.[30]

One of the main differences when comparing our study with previous work concerns the duration of diabetes in our patient groups being generally shorter than in other studies. In this context it is reassuring that evidence exists to support the prolonged maintenance of normal fibrinolysis in the setting of uncomplicated disease.[24,31]

It seems safe to conclude that the impairment of fibrinolysis associated with smoking is circumvented in Type 1 DM; that levels of the principal activator and inhibitor of fibrinolysis are normal in uncomplicated Type 1 DM, and that breakfast and exogenous insulin do not significantly alter t-PA or PAI-1 levels.

The response to venous occlusion was essentially normal in our patients with diabetes, consistent with findings elsewhere.[24,28,29,32] Our findings suggest that smoking does not significantly compromise non-specific endothelial cell function in diabetes prior to the development of advanced complications.

Surprisingly little information has previously been generated regarding platelet PAI-1. We found that the platelet pool of PAI-1 was quantitatively normal in smokers and non-smokers with diabetes, echoing the resistance of the platelet pool to change in a variety of conditions.[33]

We previously described the effects of chronic smoking in a healthy population, and found that fibrinolytic potential in plasma was significantly impaired in smokers due to an elevation in PAI-1,[8] in support of other published work.[34,35] The protocol for the current study was virtually identical in all respects to that employed in our population without diabetes, with the exception that patients with diabetes were allowed their usual breakfast and insulin on the morning of study. The groups of smokers and non-smokers with diabetes described here, and the respective groups without diabetes, were extremely similar with regard to baseline characteristics.[8] The previously described healthy non-smokers (in whom fibrinolysis can be considered normal) and healthy smokers therefore represent closely matched healthy control groups for the non-smokers and smokers with diabetes, respectively. A summary of data from the subjects without diabetes[8] and patients with diabetes is shown in Table 3.

The lack of association between smoking and reduced fibrinolytic potential in patients with Type 1 DM, contrasting with the pattern in subjects without diabetes, suggests a critical role for insulin secretion in mediating increased PAI-1 in smokers without diabetes. Smoking

Table 2. T-PA and PAI-1 in plasma and platelets

	Non-smokers	Smokers
Plasma t-PA antigen (ng ml^{-1})		
Before VO	4.4 (1.5–8.3)	3.8 (1.5–8.3)
After VO	19.2 (6.9–64.2)	15.6 (5.6–59.4)
Plasma PAI-1 antigen (ng ml^{-1})		
Before VO	10.2 (2.5–87.4)	14.3 (2.8–52.0)
After VO	13.9 (4.6–153.6)	20.2 (6.5–71.8)
Plasma t-PA:PAI-1 ratio		
Before VO	0.44 (0.07–2.60)	0.36 (0.07–1.00)
After VO	1.32 (0.34–6.80)	0.97 (0.18–2.15)
PAI–1 per platelet [ng PAI-1 (10^6 platelets)$^{-1}$]	0.34 (0.15–1.39)	0.31 (0.14–0.92)

Results expressed as median (range). VO, venous occlusion.

Table 3. T-PA and PAI-1 levels: comparison of levels in smokers and non-smokers with or without diabetes

	Non-smokers with diabetes	Smokers with diabetes	Non-smokers without diabetes	Smokers without diabetes
n	18	18	18	18
Plasma PAI-1	10.2[b]	14.3[b]	14.2[a]	34.6
(ng ml^{-1})	2.5–87.4	2.8–52.0	3.6–83.1	3.8–182.5
Plasma t-PA	4.4	3.8[a]	5.0	6.2
(ng ml^{-1})	1.5–8.3	1.5–8.3	1.6–17.5	1.6–24.6
Plasma t-PA:PAI-1	0.44[c]	0.36[a]	0.30	0.16
ratio	0.07–2.60	0.07–1.00	0.08–1.62	0.04–0.67
Platelet PAI-1	0.34	0.31	0.38	0.39
[ng PAI–1 (10^6 platelets)$^{-1}$]	0.15–1.39	0.14–0.92	0.13–0.67	0.10–0.92

Results are expressed as median and range. For any given parameter measured, the only statistically significant differences were found when comparing 'smokers without diabetes' with other groups.
[a]$p<0.05$ compared with 'smokers without diabetes'; [b]$p<0.005$ compared with 'smokers without diabetes'; [c]$p<0.001$ compared with 'smokers without diabetes'.
Data relating to subjects without diabetes has been reported previously,[8] as described in the Discussion.

is recognized to cause insulin resistance,[36,37] and thus hyperinsulinaemia, in subjects without diabetes. Against this background, smoking can stimulate triglyceride production,[38] and multivariate analysis has shown that plasma PAI-1 levels correlate with VLDL concentrations rather than insulin concentration or measures of insulin sensitivity.[39,40] In the population with diabetes, as was found in the population without diabetes,[8] triglycerides correlated with plasma PAI-1 in smokers, but not in non-smokers. However it seems unlikely that triglycerides are solely responsible for the elevation in PAI-1 in smokers without diabetes, as no significant difference was found between triglyceride levels in smokers with and without diabetes, while PAI-1 levels were significantly higher in subjects without diabetes (Table 3). From the available evidence it may be inferred that smoking, via triglycerides/VLDLs, induces a low level of PAI-1 release which, in subjects without diabetes, is significantly augmented (either directly, or indirectly via VLDLs or triglycerides) by insulin resistance/hyperinsulinaemia. This hypothesis contends that such augmentation cannot be effected in diabetic subjects, in whom endogenous insulin release is conspicuously absent.

In conclusion we consider that in the absence of advanced complications, circulating fibrinolytic potential is normal in Type 1 DM, and that this situation is not altered by chronic smoking. The radically different response to smoking in patients with diabetes from that in smokers without diabetes offers important potential insights into the regulation of PAI-1 release.

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